Plant molecular biology

Tailoring crop improvement

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GENE manipulation techniques are likely to result in great improvements in crop production. Successful first steps towards introduction of disease resistance and herbicide resistance in plants were reported at a recent meeting*.

Cross-protection, inoculation of plants with mild strains of viruses, is often used to reduce losses due to more virulent strains, such as tobacco mosaic virus. To elucidate the molecular mechanism of resistance to tobacco mosaic virus infection, a chimaeric gene containing a cloned complementary DNA of the virus coat protein under the control of the cauliflower mosaic virus 35S transcript promoter has been introduced into tobacco cells on a tumour-inducing plasmid of *Agrobacterium tumifaciens*. The transfected plants produce high levels of the virus coat protein (R. Beachy, Washington University, St Louis; ref. 1). Protection from the virus, manifested as a marked delay in the appearance of symptoms, is dosage-dependent and can be partially overcome by inoculation with naked viral RNA. The number of primary sites of infection may be reduced when the virus is encapsulated by the coat protein, and the limited amount of protection to naked viral RNA suggests that there is a second mechanism affecting viral replication or spread. It will be of interest to see whether this strategy for engineering protection will be useful in other plants.

There has been progress in improving resistance of some plants to the broad-spectrum herbicide glyphosate, which inhibits the enzyme 5-enolpyruvyl shikimate 3-phosphate synthase (ESPS). A mutant bacterial enzyme which is insensitive to glyphosate inhibition confers some resistance to the herbicide when expressed in the cytoplasm of transgenic plantlets[2,3]. The plant enzyme is chloroplastic, and by expression of a chimaeric gene comprising transit peptide sequences of the small subunit (*ssu*) gene of ribulose bisphosphate carboxylase fused to the glyphosate-insensitive bacterial gene, the resistant ESPS can be made to accumulate rapidly in chloroplasts of transgenic plantlets (G. della Cioppa, Monsanto, St Louis; ref. 4). These plantlets are 1,000-fold less sensitive to the herbicide than those in which the transgene product accumulates in the cytoplasm.

Transgene regulation and protein targeting will be crucial for effective engineering, and new data indicate that 5′ flanking regions are not the only place where *cis*-acting gene regulatory sequences act. In petunia, the ribulose bisphosphate carboxylase small subunit is encoded by a family of eight genes that exhibit markedly different levels of expression. Analysis of the expression of transgenes comprising 5′ flanking, coding and 3′ flanking regions of different *ssu* genes indicates that sequences within the gene and 3′ flanking region have a major impact on expression (C. Dean and J. Bedbrook, Advanced Genetic Sciences, Inc.). Similarly, in chimaeric gene constructs containing the chloramphenicol acetyl transferase (*cat*) reporter gene and various promoters, the first intron from the maize alcohol dehydrogenase-1 (*Adh-1*) gene has a great impact on expression in electroporated maize protoplasts (V. Walbot, Stanford University). Expression of *cat* increased nearly 200-fold with the weakest promoter tested, 60-fold with the *Adh-1* promoter, and only 7-fold with the cauliflower mosaic virus 35S promoter, the most efficient promoter tested.

Future prospects depend on the development of an increased repertoire of gene transfer methods and systems, as well as the identification of plant genes that are agriculturally significant targets for manipulation. It is striking that only one plant receptor has so far been cloned — the photoreceptor phytochrome, involved in morphogenetic and tropic responses[5,6]. Two isoforms of phytochrome have been identified in etiolated plants. This polymorphism, as well as differences identified between phytochromes in etiolated and in green plants[7], could have considerable functional significance. The different polypeptides seem to arise from differential expression of members of a small gene family, although alternate translation of a single transcript, as well as post-translational modification of the proteins, may also be involved (P. Quail, University of Wisconsin, Madison). The au^{w} mutant of tomato exhibits many features of etiolated plants, and protein blot analysis indicates that the level of a phytochrome polypeptide is very low in the mutant. This system offers the potential for studying the relationships between structure and function of this receptor by examination of the effects of specific mutations engineered *in vitro* on phytochrome levels in transgenic plants following re-insertion of the modified genes, and may provide opportunities for precise engineering of changes in day-length requirements and response to shading of target crop plants.

Arabidopsis thaliana, the common wall cress, possesses several attractive features (ref. 8 and discussed in *News and Views*[9]). Its generation time of 4–5 weeks, the ease with which it may be propagated in large numbers, its lack of repetitive sequences and small genome size (about 70,000 kilobase pairs)[10] are advantageous for the rapid identification and cloning of mutated plant gene sequences. C. Somerville (Michigan State University) produced three *Arabidopsis* plants resistant to sulphonylurea by screening 300,000 mutagenized seedlings[11]. In collaboration with J. Smith and B. Mazur (DuPont), a yeast acetolactase synthetase gene was used to identify the gene encoding the *Arabidopsis* enzyme in a genomic library generated from one of these sulphonylurea-resistant plants. Somerville also identified *Arabidopsis* mutants that are defective in lipid biosynthesis[12]. Mutants for many of the biosynthetic steps, including most of the desaturase steps, can be identified by gas chromatographic analyses of leaf tissue derived from mutagenized plants. When these data are combined with two-dimensional gel analyses of proteins, it will be possible to identify proteins that are differentially expressed in the mutants and to clone the genes of this pathway.

P. Ahlquist (University of Wisconsin, Madison) described the designed modification of brome mosaic virus for use as a vector[13]. Extremely high levels of expression can be achieved with this gene transfer system. Milligramme quantities of virion coat protein are produced in infections with the wild-type virus. With engineered constructs, *cat* activity already has been obtained which is five- to fifteen-fold greater than that achieved with tumour-inducing plasmid-based vectors, and may be further increased by control of messenger RNA and/or protein degradation. Future potential advantages of this viral vector are the broad range of monocot and dicot hosts as well as effective gene transfer to vegetative cells of perennial crop plants. □

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